Journal of Cellular Biochemistry Supplement 24:24–31 (1996)

Cell Culture Methods for the Establishment of the NCI Series of Lung Cancer Cell Lines

Herbert K. Oie, Edward K. Russell, Desmond N. Carney, and Adi F. Gazdar

NCI-Navy Medical Oncology Branch, National Cancer Institute and the National Naval Medical Center, Bethesda, Maryland 20814 (H.K.O., E.K.R.); Department of Medical Oncology, Mater Misericordiae Hospital, Dublin 7, Ireland (D.N.C.); The University of Texas, Southwestern Medical Center of Dallas, Dallas, Texas 75235-8593 (A.F.G.)

Abstract More than 200 human small cell lung cancer and non-small cell lung cancer cell lines were established over 15 years mainly by utilizing the serum-free, hormone and growth factor supplemented, defined media HITES and ACL4. Use of modified, established cell culture techniques such as the mechanical spillout method for the releasing of cell aggregates from tumor tissue, ficoll gradient centrifugation for the separation of tumor cells from erythrocytes and tissue debris, and an apparatue consisting of a platinum tubing attached to a suction flask for removal of spent medium have greatly contributed to the success in culturing tumor cells. Characterization of these lung cancer cell lines have extended our knowledge of lung cell biology. Studies elucidating the nutritional requirements of lung cancer cell growth may be helpful for the manipulation of these tumors in patients.

Key words: lung cancer cell lines, cell culture techniques, SErum-free, defined medium

We have devoted more than 15 years to the cultivation and establishment of human lung cancer cell lines with the following goals in mind: first, to expand the tumor cell population from clinical specimens so that drug and radiation sensitivity data can be obtained and used for individualized patient therapy; second, to develop a panel of cell lines representing the various cell types that comprise small cell (SCLC) and non-small cell (NSCLC) lung cancers; third, to determine the hormonal and growth factor requirements of each tumor cell type to better grow these cells directly from clinical specimens and possibly to use nutritional requirements to manipulate these tumors in patients; and finally, to extend our general knowledge of the biology of lung cancer.

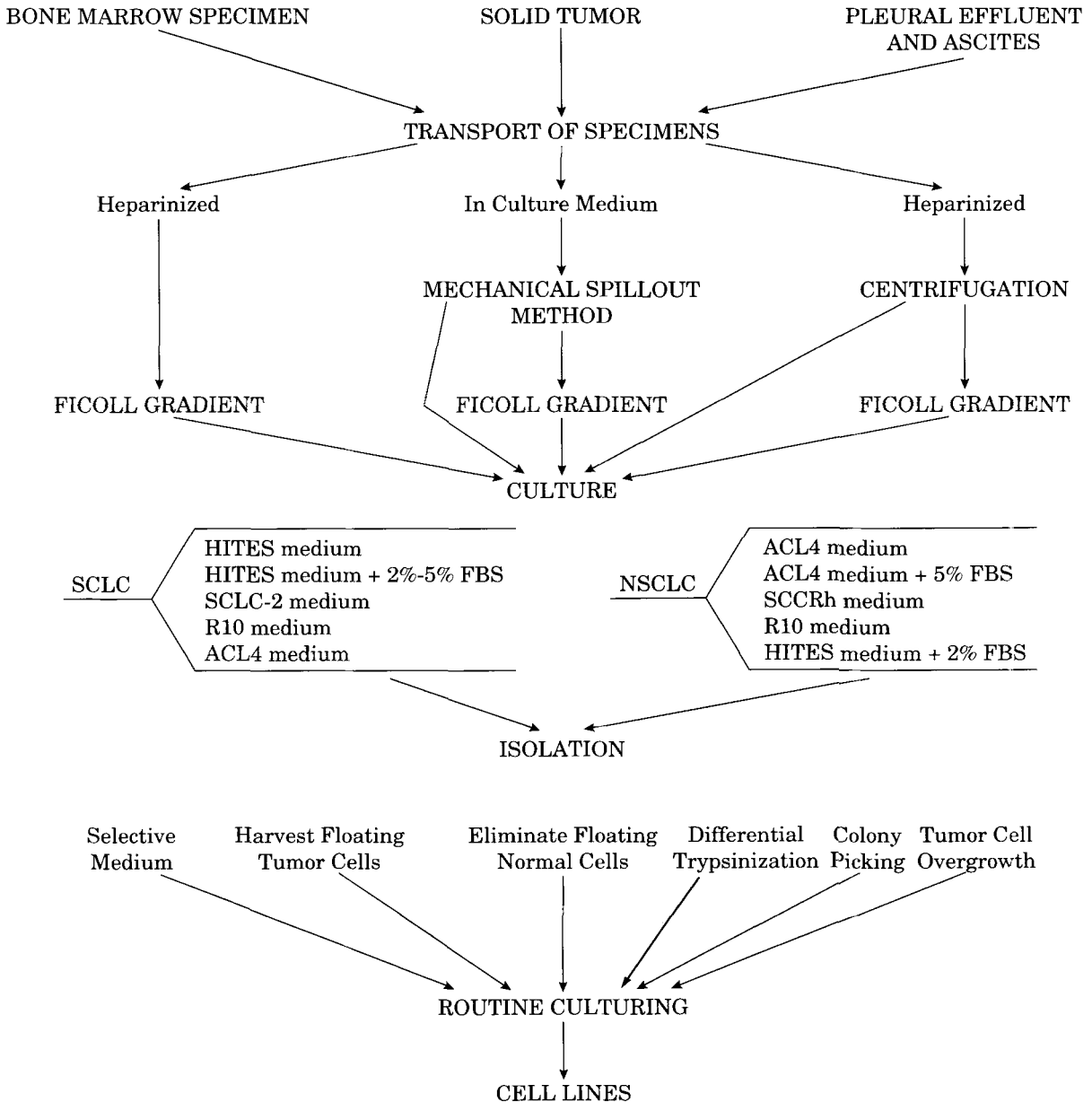
In the process of culturing lung cancer cells, we noticed that tumor cells grew better in patients than in culture. In fact, normal fibroblasts generally grew faster and overgrew cancer cells. Deficiency in growth factors or presence of tumor cell growth inhibitors in serum-supplemented medium could explain the poor in vitro growth of lung cancer cells [1]. To eliminate the problems created by adding serum to medium, serum-free, hormone- and growth factor-supplemented, defined media were developed. In the late 1970s, led by G. Sato's laboratory, serum-free, defined media for the cultivation of many different types of cells were reported [2–4]. In our laboratory, the serum-free, defined media HITES for the selective growth of SCLC [5] and ACL4 for the culturing of adenocarcinoma [6] were developed. These two media significantly contributed to the success we have had in growing SCLC and adenocarcinoma of the lung [7–9]. The use of growth factor-supplemented, serum-free media for the propagation of SCLC and lung adenocarcinoma has served as an impetus to determine the growth factor requirements for the selective growth of other lung cancer cell types.

In addition to improved media, new and innovative ways of handling and processing tumor specimens have been continuously tested to improve the culturing of all types of cells. Detoxifi-

Received January 19, 1996.

Address reprint requests to Herbert K. Oie, NCI-Navy Medical Oncology Branch, National Cancer Institute and the National Naval Medical Center, Bethesda, Maryland 20814.

TABLE I. Schema for the Cultivation of Human Lung Cancer Cells



cation reagents such as polyvinyl-pyrollidone and methylcellulose have been added to specimen-transporting medium and growth medium to neutralize the cell damaging effects of enzymes released by dead or dying cells [10]. Because most cells require substrate-adhesion to proliferate, flasks coated with attachment factors such as collagen, laminin, fibronectin, poly-D-lysine, fetuin, and biomatrices have been tested to improve adhesion and consequently cell growth [6,11,12]. The less traumatic enzymes, collagenase [10,13] and polymyxa protease [14,15], are replacing trypsin not only for disaggregating tumor tissue but also for routine passaging of cell cultures.

In this paper, we describe in some detail the procedures we have developed over the years, using a variety of techniques for the cultivation, isolation, and establishment of more that 200 SCLC and NSCLC cell lines, the NCI Series of Lung Cancer Cell Lines. The procedures used for each of the 3 types of specimens are diagrammatically summarized in Table I. The hormone- and growth factor-supplemented media used for the culturing of SCLC and NSCLC cells are shown in Table II.

TABLE II. Hormone- and Growth Factor-Supplemented Media for the Cultivation of Human Lung Tumor Cells

	HITES	SCLC-2	ACL-4	SCCRh
Basal medium	RPMI 1640	RPMI 1640	RPMI 1640	DMEM/HAM'S F-12 (1:1 mixture)
Supplement				
Insulin	5 μg/ml	5 μg/ml	20 μg/ml	
Transferrin	10 μg/ml	10 μg/ml	10 μg/ml	
Sodium selenite	30 nM	30 nM	25 nM	
Hydrocortisone	10 nM	10 nM	50 nM	1 μM
17-B-estradiol	10 nM	10 nM		
Epidermal growth factor			1 ng/ml	10 ng/ml
Triiodothyronine			0.1 nM	
Ethanolamine		10 μM	10 μM	
Phosphorylethanolamine		10 μM	10 μM	
Cholera toxin				1 ng/ml
Arginine vasopressin		10 ng/ml		
Bombesin		0.1 μM		
Sodium pyruvate			0.5 mM	
Bovine serum albumin		0.2%	0.2%	
HEPES	10 mM	10 mM	10 mM	10 mM
Glutamine	2 mM	2 mM	2 mM	2 mM
Fetal bovine serum				5%

METHODS, RESULTS, AND COMMENTS

Procurement of Tumor Specimens

Solid tumors (lung mass, tumors in lymph nodes and other extra-pulmonary sites) were surgically resected and transported to Pathology where a pathologist examined, selected, and excised a sample for transport to the Cell Culture Laboratory in RPMI 1640 medium.

Many solid tumors are necrotic. Since dead cells released enzymes which can destroy cell viability and since specimens are not always handled aseptically in Pathology, addition of antibiotics to the transporting medium and carrying the specimens on ice are precautionary measures which could insure the deliverance of uncontaminated, viable tumor specimens to the laboratory.

Pleural and ascitic effluents were brought to the laboratory directly from the collection sites. Heparin (5 μg/ml) was added to the effluents to prevent clotting.

Bone marrow aspirates were collected at the Bone Marrow Laboratory in heparinized tubes and hand-carried to the laboratory soon after collection.

Tumor specimens sent to the laboratory for cultivation by outside institutions were packed in ice and sent by overnight service.

Processing of Tumor Specimens

Most specimens were processed soon after arrival (usually within 30–60 min). However, specimens which could not be processed right away were stored in the refrigerator. On some occasions, tumor samples had to be kept overnight in the refrigerator. Many of these specimens still yielded viable cells when processed the following morning.

Routinely, for each specimen received in adequate quantities a sample was taken for L-dopadecarboxylase assay, several provials of tissue were frozen dry and in cryoprotective medium (40% RPMI 1640, 10% dimethyl sulfoxide, 50% fetal bovine serum), and an aliquot of cell suspension was fixed in Saccomonno solution for cytological examination. All cells were initially cultured in media containing antibiotics (penicillin, 100 U/ml; streptomycin, 100 μg/ml; gentamicin, 5 μg/ml; fungizone, 5 μg/ml).

Bone marrow specimens. Usually 2–4 ml of bloody aspirate was received from the Bone Marrow Laboratory. Discontinuous Ficoll gradients (Lymphocyte Separation Medium [LSM], density at 20°C: 1.077–1.088 gm/ml) were used to concentrate tumor cells and eliminate erythrocytes. Two milliliter aliquots of aspirates were transferred to 15 ml conical centrifuge tubes,

diluted 4–5 times with RPMI 1640 medium, and mixed thoroughly. After carefully depositing 2 ml Ficoll in the bottom of each tube, the samples were centrifuged in a refrigerated table top centrifuge (Beckman GPR Centrifuge, Beckman, Fullerton, CA) at 1,500 rpm for 15 min at 4°C. The interface layer was harvested with a pipet and transferred to a new 15 ml centrifuge tube. The cells were washed twice in 10 ml volumes of RPMI 1640 medium to remove Ficoll, recovering the cells each time by centrifugation at 1,000 rpm for 5 min. The cell pellet was resuspended in RPMI 1640 medium and seeded for culture in 25 cm^2 tissue culture flasks containing 4 ml of media.

Pleural and ascitic effluents. Processing of these effluents depended on volume and amount of cellular content, i.e., erythrocytes, mesothelial cells, monocytes, tumor cells, etc. For specimens with little or no erythrocytes, the cells were centrifuged, resuspended in RPMI 1640 medium and without further processing distributed into 25 cm^2 flasks.

For specimens with large amounts of erythrocytes, the cells were centrifuged, resuspended in RPMI 1640 medium, transferred to 15 or 50 ml conical centrifuge and separated by Ficoll gradients. The interface layer was harvested, washed twice in RPMI 1640 medium, and seeded into 25 cm^2 flasks. The pellets from the gradients frequently contained a significant amount of tumor aggregates. After a single wash, the pellets were resuspended in RPMI 1640 medium and left standing in a rack at room temperature, allowing the heavier cell aggregates to sediment by gravity. After aspirating off the supernatant fluid containing most of the erythrocytes, the cell aggregates were cultured.

Solid tumors. The tumor specimen was placed in a sterile 100 mm Petri dish and a small amount of medium was added to keep the tissue wet. Using forceps and scissors, necrotic areas, fatty tissue, blood clots, and connective tissue were removed. The remaining tumor tissue was processed.

Several methods for disaggregating solid tissues are available. Most use mechanical means, enzymatic digestion, or a combination of the two methods. Very early in our work with SCLC, we found that trypsin digestion of solid SCLC tumors resulted in poor recovery of viable cells. This method also produced a lawn of tumor and normal cells uniformly distributed over the flask surface. Under this condition, the faster growing normal cells, mainly fibroblasts, easily outgrew and overgrew the slower growing carcinoma cells.

The simplest, fastest, and least traumatic method of obtaining cells for culture was by the mechanical spillout method [10]. The tumor was minced into very small pieces with sterile scissors or by cross-cutting with two scapels. Tumor aggregates usually adhered loosely to stromal tissue and were released into the medium by mincing and washing. Tumor aggregate-containing washes were harvested, pooled, and centrifuged. Cell suspensions with too much erythrocytes were subjected to Ficoll gradient separation to eliminate erythrocytes, dead cells, and other tissue debris. Otherwise, the cell aggregates were seeded into 25 cm^2 flasks. The remaining minced tissue pieces were either processed further or put directly in culture (usually into 75 cm^2 flasks).

The mechanical spillout method minimized stromal cell contamination since these cells were not easily dislodged from the tissue matrix by mechanical means. The tumor cells when seeded as aggregates established colonies of cells (instead of single, isolated cells) which were better able to compete for survival with faster growing stromal cells, especially fibroblasts.

Our experiences over the years showed that the chances of growing tumor cells from certain specimens were very poor. Tough, whitish tissue which was difficult to mince contained mainly fibrous tissue and yielded very little if any tumor cells. Specimens which produced creamy, yellow exudate when squeezed with forceps contained mostly necrotic material. Although the exudate contained cells, very few of these cells were viable.

Culture and Isolation

Small cell lung cancer. SCLC were grown in HITES, HITES plus 2–5% FBS, SCLC-2 [16], RPMI 1640 plus 10% FBS (R10), and ACL4 plus 5% FBS, in that order of priority, depending on the availability of cells. A very small percentage of SCLC cells grew poorly in HITES medium with or without serum supplement, but proliferated well in SCLC-2. For in vitro growth, these cells probably needed bombesin, cholera toxin, or epidermal growth factor which are found in SCLC-2.

Since most SCLC cells grew as "floating aggregates" while normal cells required substate-adhesion to proliferate, the isolation of SCLC

was relatively simple. By harvesting and transferring the floating aggregates to new flasks, pure SCLC cultures were easily achieved.

SCLC cells which grew as adherent colonies among stromal cells usually did not grow firmly attached to the flask surfaces. By gently rapping the flasks, parts of colonies or even whole colonies of SCLC cells could be dislodged, harvested, and transferred to new flasks. After a few such manipulations, SCLC cultures free of stromal cells were obtained.

The development of the serum-free, growth factors supplemented, defined medium HITES proved to be a boon to SCLC cultivation. Not only did most SCLC cells grow in this medium but the growth of stromal cells was selectively inhibited. This eliminated the need to use other methods for separating tumor cells from normal cells. It was only after the development and use of HITES medium for the culture of SCLC cells that we had increased success in isolating and establishing SCLC cell lines. Whereas only six lines were established from SCLC specimens put into culture in the 3 years (1976–78) preceding the development of the HITES medium, twenty-three lines resulted from the cultures started during the 3 years (1979–81) after we began routinely using the HITES medium for the cultivation of SCLC tumor cells.

HITES medium proved especially useful in eliminating proliferating B-lymphocytes present in some SCLC specimens. Both SCLC and B-lymphocytes grew as floating aggregates. However, B-lymphocytes required serum for proliferation so did not grow in serum-free HITES medium. Since B-lymphocytes can be considered "normal" counterparts of tumor cells, efforts were made to isolate B-lymphocytes whenever these cells were found in tumor samples. By culturing mixed suspensions of tumor and B-lymphocytes in serum-containing media such as ACL4 plus 5% FBS and SCCRh in which most SCLC cells did not grow, B-lymphocytes were isolated and eventually established as cell lines. B-lymphocytes were also found in NSCLC samples. In this case, isolation was easier since most NSCLC cells grew attached to the flask surfaces. The separation was achieved by harvesting the floating aggregates of B-lymphocytes and transferring the cells to new flasks. Pairs of B-lymphocytes and tumor cell lines from the same patient proved to be valuable in studies comparing the properties of tumor and normal cells.

The addition of serum to defined media such as HITES and ACL4 resulted in a diminution of their selective capabilities, allowing normal cells as well as other tumor cell types to proliferate. However, serum also enhanced the growth of the tumor cells which could grow in the serum-free, defined medium. In addition, tumor cells that grew poorly in serum-free medium, usually grew better in the same medium supplemented with serum, suggesting that the serum-free medium was deficient in growth factor(s) which was supplied by serum.

Routinely, the tumor samples were started in media containing antibiotics. Soon after isolation, the cultures were fed with antibiotics-free medium so that the cultures could be tested for mycoplasma (earlier done by culturing the samples on special agar plates and by the Hoechst stain method, Microbiological Associates, Bethesda, MD; more recently by the Gen-Probe nucleic acid hybridization assay). Also, as soon as possible, several provials of each tumor cell isolate were frozen in cryoprotective medium.

Cells grown in vitro for many passages, especially in serum-free medium, frequently undergo a "crisis." The cells grew poorly with more cells dying than proliferating. This cell culture event became quite apparent early in our attempts to establish SCLC cell lines in HITES medium. Fortunately, it was found that addition of large amounts of FBS (20% or more) to the growth medium usually helped the cultures overcome this crisis. Cultures which survived this crisis frequently developed into continuous cell lines. Thereafter, to prevent loss of cell cultures to crisis, a few flasks from each specimen were grown in serum-containing medium after isolation.

Non-small cell lung cancer. The culturing of NSCLC cells presented a more complex problem than growing SCLC cells, since NSCLC comprised at least three major cell types (large cell carcinoma, adenocarcinoma, and squamous cell carcinoma) in addition to several subtypes. Adding to the problem was the occurrence of NSCLC tumors containing multiple tumor cell types.

NSCLC samples were cultured in ACL4, ACL4 plus 5% FBS, SCCRh [17], R10, and HITES plus 2% FBS, in that order of priority, again depending on the availability of cells. In vitro cell cultivation is far from being an exact science. Many of the day-to-day decisions during our early attempts at culturing NSCLC cells relied on trial

and error, intuition, and the limited experiences gained culturing SCLC cells. As was the case with HITES medium, ACL4 medium played a significant role in the successful cultivation of adenocarcinoma. NSCLC specimens cultured during the first 7 years (1976–82) prior to the development of ACL4 medium yielded seven adenocarcinoma lines. In contrast, twenty-four adenocarcinoma cell lines were established from cultures started during the next 4 years (1983–86) when ACL4 medium was routinely used for culturing tumor specimens.

Since SCCRh medium contained 5% FBS, it was not as effective in selectively growing squamous cell carcinoma. Addition of serum to ACL4 medium enhanced the growth of adenocarcinoma cells but also allowed proliferation of normal cells. By initially starting cultures in serum-free ACL4 medium, normal cell growth was inhibited (not eliminated) and gave the slower growing tumor cells a better start. After the tumor cells were fairly well established in culture, addition of serum helped to enhance tumor cell growth.

With NSCLC samples, separation of tumor cells from normal cells was a major problem, since both cells usually grew as adherent colonies. In some cases of heavily seeded cultures, tumor aggregates attached to the flask within 24 h, so that removal of the media greatly reduced the amounts of contaminating stromal cells and erythrocytes. In other cases, especially with pleural effluents, the faster attaching cells were normal cells (mesothelial cells, moncytes, etc.) so that the medium contained tumor cells and a reduced number of normal cells and erythrocytes. In either case, a series of these manipulations usually produced fairly contaminant-free tumor cell cultures.

In most NSCLC cultures, adherent colonies of tumor cells and normal cells were randomly distributed over the flask surfaces. A number of techniques were utilized for the isolation of tumor cells. In one technique, cells from well-isolated, large colonies were harvested by completely removing the medium, placing a drop of medium on the selected colony, dislodging the tumor cells into the medium by scraping with a Pasteur pipet, aspirating the detached cells into the pipet, and depositing the cells into wells of a 24- or 96-well plate. We have found that small numbers of cells suspended in small volumes of medium survive best in small containers.

Another technique took advantage of the differences in cell susceptibility to trypsinization. By controlled exposure of adherent mixed tumor cell/normal cell cultures to diluted trypsin (1:10 dilution), selected detachment of either tumor cells or normal cells (usually fibroblasts) could be achieved. Trypsinates containing mainly normal cells were discarded and the culture flask containing the tumor cells was refed with growth medium. Trypsinates containing mainly tumor cells were centrifuged. The cell pellet was washed twice in RPMI 1640 and cultured in new flasks. The old flask containing normal cells was discarded.

Despite being completely surrounded by fibroblasts, some adenocarcinoma colonies continued to grow. Since lateral growth was impeded by surrounding fibroblasts, continued growth was manifested as aggregates of cells loosely attached to the tumor cell colonies. These aggregates were easily dislodged by gentle rapping of the flask, harvested, and cultured in new flasks.

Some large cell carcinoma and squamous cell carcinoma colonies, completely surrounded by fibroblasts, formed densely packed colonies which continued to grow laterally, slowly infiltrating and displacing the adjacent fibroblasts. Eventually, the entire flask could be taken over by these tumor cells. Since this occurrence could take months, maintenance of these cultures required patience and careful handling so as to avoid contamination with bacteria and fungi.

Following the successful isolation of tumor cells, the cultures were routinely passaged every 7–10 days. Usually, it was desirable to keep the cultures in the original isolation media to prevent the possibility of any medium-determined changes in cell properties or selective growth of a subtype present in the culture in undetected numbers. Early passage cells were periodically frozen in cryoprotective medium to prevent loss of the cell lines through contamination or other laboratory accidents. The frozen cell lines were stored in at least two separate ultra low temperature freezers (−135°C or lower, nitrogen or compressor driven) kept in different locations to insure that the cell lines will not be lost due to freezer breakdowns. Only after a cell culture had been in continuous culture for 6–12 months and/or 20–25 passages was it considered a cell line.

All cell lines were eventually adapted to grow in RPMI 1640 plus 10% FBS to make maintenance over long periods easier. Since many re-

searchers who request cell lines from this laboratory use RPMI 1640 medium supplemented with serum to grow their cells, it was more convenient to send to them cell lines that were already adapted to grow in this medium.

DISCUSSION AND CONCLUSION

Several developments have contributed to the progressive success we have had in cultivating and establishing large numbers of human lung cancer cell lines. A suction apparatus fitted with a 9–12-inch-long platinum tubing for the sterile aspiration of media and other fluids has facilitated the handling of large numbers of cultures. Since platinum heats and cools rapidly, it proved to be the ideal material for this tubing. Although platinum is expensive, the time, effort expended, and supplies (pipets, centrifuge, etc.) saved by using this apparatus easily made up for the cost of the platinum tubing. If properly cared for, platinum tubings can last for a long time.

The early recognition that few viable cells were recoverable by disaggregating solid SCLC tumors by trypsinization led to the use of the mechanical spillout method, not only for the processing of SCLC specimens, but for all other solid tumors. Many of our techniques for growing, isolating, and maintaining cells were developed for cultures started from aggregates, not single cell suspensions.

Serum-free, completely or partially defined, selective medium was probably the single most important development which led to our success in culturing SCLC and NSCLC cells. The selective proliferation of tumor cells and inhibition of normal cell growth eliminated the most common cause of failure to establish cell lines, overgrowth by fibroblast cells.

Not only has HITES medium and ACL4 medium been useful in developing SCLC and NSCLC cell lines, respectively, but other human tumor cell lines have been established in these media. An adrenocortical carcinoma NCI-H295 was started in HITES medium [18]. A myeloma cell line NCI-H929 [19] and several colorectal cell lines [20] were established in serum-free ACL4 medium or serum-supplemented ACL4 medium. Park et al. [21] in 1990 reported establishing cell lines from a wide cariety of human cancers including lung cancer, myeloma, melanoma, pancreatic cancer, hypernephroma, and laryngeal cancer in ACL4 medium supplemented with 5% FBS. More recently, Masuda et al. [15] in 1991 succeeded in establishing 20 human NSCLC cell lines (16 adenocarcinoma, 2 large cell carcinoma, 1 squamous cell carcinoma, and 1 malignant mesothelioma) in ACL4 medium. Similar nutritional requirements for in vitro growth appear to be shared by a wide variety of human tumor cell types.

Few squamous cell carcinoma cell lines are available. One reason could be the lack of a serum-free, defined medium for the culturing of this cell type. Failure to prevent replicating squamous cells from terminally keratinizing may be the most probable reason for not being able to establish continuous squamous cell lines [22]. More attention should be focused on two other interesting lung cancers, carcinoids and mesotheliomas.

Finally, extensive characterization of a large number of SCLC and NSCLC cell lines established in this laboratory has greatly increased our knowledge of lung cancer. With the ever-improving techniques for cell culturing and the rapid accumulation of nutritional requirements for many different cell types, it is not inconceivable that cell lines from most human tumor cell types should soon become available for study.

ACKNOWLEDGMENTS

The authors thank Harold Stull, Virginia Bertness, Sylvia Stephenson, Harvie Sims, Alfreda Simmons, Dr. Elizabeth Simms, Dr. Martin Brower, and Dr. Fabian Calvo for their contributions to the development and testing of the methods employed in the culturing and establishing of the NCI Series of Lung Cancer Cell Lines.

REFERENCES

1. Barnes D, Sato G (1980): Serum-free cell culture: A unifying approach. Cell 22:649–655.
2. Hayashi I, Sato G (1976): Replacement of serum by hormones permits the growth of cells in a defined medium. Nature 259:132–134.
3. Bottenstein J, Hayashi I, Hutchings S, Masui H, Mather J, McClure DB, Ohasa S, Rizzino A, Sato G, Serrero G, Wolfe R, Wu R (1979): The growth of cells in serum-free hormone-supplemented media. In Jakoby WB, Paston IH (eds): "Methods in Enzymology." New York: Academic Press, vol. 58 pp 94–109.
4. Barnes D, van der Bosch J, Masui H, Miyazaki K, Sato G (1981): The culture of human tumor cells in serum-free medium. In Pesika S (ed): "Methods in Enzymology." 79. New York: Raven, vol. 79 pp 369–399.
5. Simms E, Gazdar AF, Abrams PG, Minna JD (1980): Growth of human small cell (oat cell) carcinoma of the lung in serum-free growth factor supplemented medium. Cancer Res 40:4356–4363.

6. Brower M, Carney DN, Oie HK, Gazdar AF, Minna JD (1986): Growth of cell lines and clinical specimens of human non-small cell lung cancer in a serum-free defined medium. Cancer Res 46:798–806.
7. Minna JD, Carney DN, Oie H, Bunn PA, Gazdar AF (1982): Growth of human small cell lung cancer in defined medium. In Pardee A, Sato G (eds): "Growth of Cells in Hormonally Defined Medium." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, pp 627–639.
8. Carney DN, Brower M, Bertness V, Oie HK (1984): Selective growth of human small cell lung cancer lines and clinical specimens in serum-free medium. In Barnes DW, Sirbasku DA, Sato G (eds): "Methods for Serum-Free Culture of Epithelial and Fibroblast Cells." New York: Alan Liss, Inc., pp 57–71.
9. Ruckdeschel JC, Oie HK, Gazdar AF (1986): In vitro characterization of non-small cell lung cancer. In Hansen HH (ed): "Lung Cancer: Basic and Clinical Aspects." Boston: Martinus Nijhoff Publishers, pp 49–59.
10. Leibovitz A (1986): Development of tumor cell lines. Cancer Genet Cytogenet 19:11–19.
11. Reid LM, Jefferson DM (1984): Cell culture studies using extracts of extracellular matrix to study growth and differentiation in mammalian cells. In Mather JP (ed): "Mammalian Cell Culture." New York: Plenum Publishing Corporation, pp 239–280.
12. Kleinman HK, Klebe RJ, Martin GR (1981): Role of collagenous matrices in the adhesion and growth of cells. J Cell Biol 88:473–485.
13. Slocum HK, Pavelic ZB, Rustum YM, Creaven PJ, Karakousis C, Takita H, Greco WR (1981): Characterization of cells obtained by mechanical and enzymatic means from human melanoma, sarcoma, and lung tumors. Cancer Res 41:1428–1434.
14. Matsumoto T, Yamanaka T, Hashizume S, Irie Y, Nitta K (1975): Tissue dispersion, cell harvest and fluid suspension culture by the use of bacterial neutral protease. Jpn J Exp Med 45:377–382.
15. Masuda N, Fukuoka M, Takada M, Kudoh S, Kusunoki Y (1991): Establishment and characterization of 20 human non-small cell lung cancer cell lines in a serum-free defined medium (ACL-4). Chest 100:429–438.
16. Oie HK, Brower M, Carney DN (1984): Growth factor requirements for in vitro growth of small cell and other lung cancers in serum-free defined medium. In Becker K, and Gazdar AF (eds): "Endocrine Lung in Health and Disease." Philadelphia: Saunders, pp 469–475.
17. Ervin TJ, Beckett MA, Miller D, Rheinwald JG (1982): Clonal growth and serial passage of head and neck squamous cell carcinoma. Proc ASCO 1:195 Abstr Am Soc Clin Oncol p 195.
18. Gazdar AF, Oie HK, Shackleton CH, Chen TR, Triche TJ, Myers CE, Chrousos GP, Brennan MF, Stein CA, La Rocca RV (1990): Establishment and characterization of a human adrenocortical carcinoma cell line that expresses multiple pathways of steroid biosynthesis. Cancer Res 50:5488–5496.
19. Gazdar AF, Oie HK, Kirsch IR, Hollis GF (1986): Establishment and characterization of a human plasma cell myeloma culture having a rearranged cellular myc proto-oncogene. Blood 67:1542–1549.
20. Park JG, Oie HK, Sugarbaker PH, Henslee JG, Chen TR, Johnson BE, Gazdar AF (1987): Characteristics of cell lines established from human colorectal carcinoma. Cancer Res 47:6710–6718.
21. Park JG, Kwon NS, Noh DY, Hong IG, et al (1990): Establishment of SNU cell lines in growth factor and growth hormone supplemented medium. J Korean Cancer Assoc 22:1–13.
22. Gazdar AF, Oie HK (1986): Cell culture methods for human lung cancer. Cancer Genet Cytogenet 19:5–10.